Assays for Complexed Prostate-Specific Antigen and Other Advances in the Diagnosis of Prostate Cancer

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This review discusses advances in the area of serum and tissue markers for prostate cancer. A recently developed assay for complexed prostate-specific antigen (PSA) has been found to have better specificity than that afforded by assay of total PSA. Researchers in Austria have found that lowering the PSA cutoff point for a diagnosis of prostate cancer resulted in a significant increase in identifying men with cancer at a favorable pathologic stage. Difficulties in pathologic interpretation of tissue specimens can result in both under- and over-diagnosis of prostate cancer. When in doubt, referral to a pathologist who specializes in prostate cancer is warranted. Epidermal growth factor receptor is emerging as an important therapeutic approach not only to prostate cancer but also to breast and colon cancers.
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Key words: Prostate cancer • Diagnosis • Prostate-specific antigen • Pathology • Epidermal growth factors

Several papers presented at the 13th International Prostate Cancer Update addressed serum and tissue markers. Although it is well established that prostate-specific antigen (PSA) has revolutionized all aspects of the management of men with prostate cancer and is most useful for early diagnosis of this malignancy, the need for better markers still exists.

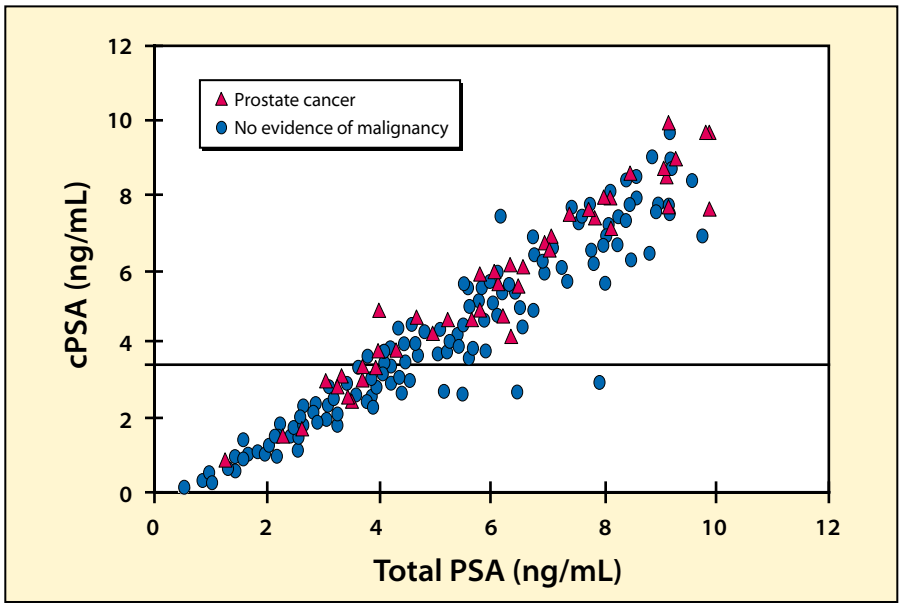

Figure 1. *Regression analysis comparing different cutoff points for complexed prostate-specific antigen (cPSA) with total PSA. Reproduced from Brawer et al[5] with permission from Elsevier Science.*

Enhanced Specificity with Complexed PSA Assay

This author provided an overview of PSA. PSA is the most important tumor marker in oncology. Widespread use of PSA assay resulted in a dramatic increase in the detection of prostate cancer in the United States, peaking in 1992. It has been established that measurement of PSA enables earlier diagnosis, and indeed this might be one of the factors associated with the decrease in prostate cancer mortality in the United States. However, there are problems with total PSA assay. It lacks sensitivity—there are false-negative results—but more importantly it lacks specificity, resulting in a high number of false-positive test results. Also, there is the overriding concern that some cancers detected in men have limited malignant potential (over-detection).

Of these limitations, the greatest problem with total PSA assay is the lack of specificity. This lack of specificity compels large numbers of men with elevated PSA levels to undergo biopsy (with its significant economic and psychological cost) only to be found, at least on the initial biopsy, free of malignancy. On the basis of this problem, a number of authors have carried out investigations of so-called "PSA derivatives," including PSA density, PSA velocity, and age-specific PSA cutoff points. Although some investigators have shown these to be promising, in most broad-based clinical practices they have proved to have little, if any, utility.

The now-recognized molecular forms of PSA have been found to have significant clinical utility. We know that once PSA gains access to the systemic circulation, the majority is complexed to protease inhibitors. Of these, the most important is α-1-antichymotrypsin. It has been established[1–3] that this form constitutes a greater proportion of the total PSA in men with malignancy. Although this has been recognized for many years, reliable assays for complexed PSA (cPSA) were lacking. We were forced to estimate the amount of cPSA by measuring the free-to-total PSA ratio. The free-to-total ratio provides essentially the same information as that gleaned by measurement of cPSA, but the latter requires only a single analyte determination. Not only is this economically advantageous, but many of the problems associated with determining the free-to-total PSA ratio, including lack of stability of the free form of PSA and the error that is caused by quotient bias when one number is divided into another, would

Figure 2. *Comparison of complexed prostate-specific antigen (cPSA) with total PSA (tPSA) specificity at fixed sensitivities. Data from Brawer et al.[6]*

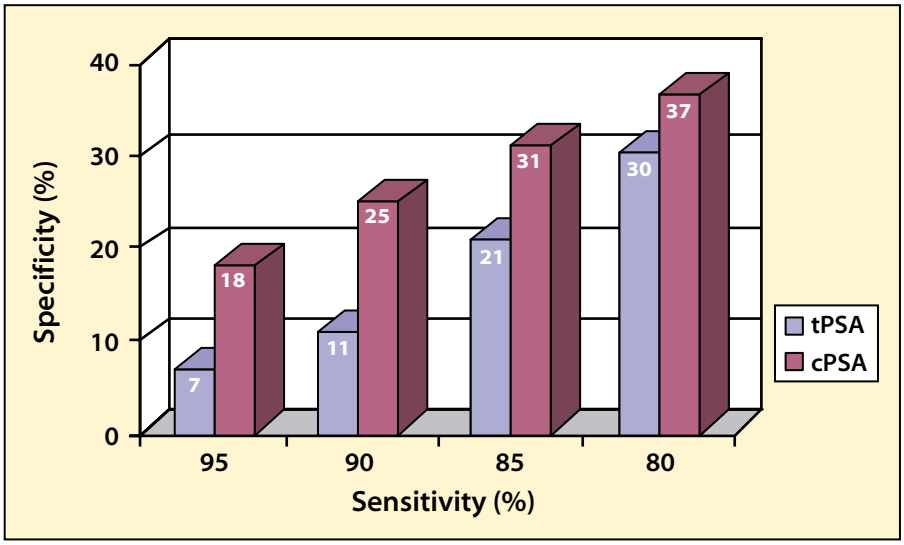

Table 1
cPSA Literature Review

Reference	N	cPSA AUC	tPSA AUC
Miller et al, 2001[7]	3000	0.539	0.522
Mitchell et al, 2001[8]	160	0.706	0.671
Brawer et al, 2000[6]	657	0.671	0.648
Okegawa et al, 2000[10]	140	0.714	0.611
Jung et al, 2000[11]	324	0.632	0.568
Filella et al, 2000[12]	251	0.873	0.851
Stamey and Yemoto, 2000[13]	170	0.568	0.519
Brawer et al, 1998[5]	300	0.722	0.688
Tanguay et al, 2002[14]	535	0.661	0.644

PSA, prostate specific antigen; cPSA, complexed PSA; tPSA, total PSA; AUC, area under the curve.

be obviated by measuring the cPSA.

Eventually, the Bayer Corporation developed a specific assay for cPSA with an automated format.[4] In our initial evaluation of this assay, we were able to demonstrate significant enhancement in test specificity, with approximately 20% improvement over that afforded by total PSA assay.[5] Figure 1 shows these results.

In a subsequent, expanded investigation,[6] we again demonstrated improvement in test performance, owing to enhanced specificity, at clinically useful sensitivity levels, as demonstrated in Figure 2. In a review of the recent literature on cPSA, most authorities have demonstrated similar results (Table 1).[6–14] One notable exception was the report by the Stanford Group.[13] They did not show any enhancement in test performance with the complexed form of PSA. Significant differences between the Stanford and our multicenter trial[6] exist. The Stanford study was smaller, and they required two sets of negative biopsies to define men as being free of cancer; but of greatest importance was the fact that the level of total PSA in the Stanford series was no different in men with or without malignancy (10.9 ng/mL in both groups). This in contrast to the multicenter trial, in which we reported total PSA of 6.0 ng/mL in men without malignancy and 8.8 ng/mL in those with cancer, a statistically significant difference.

A multicenter, prospective study that aimed to resolve this discrepancy has recently been completed.[15] This seven-site investigation included New York University, Johns Hopkins University, The Northwest Prostate Institute, The MD Anderson Medical Center, The University of Innsbruck, Cheyenne Urology, and Stanford University. A total of 830 men undergoing ultrasound-guided biopsy were included. Table 2 shows the demographic characteristics of men with and without malignancy. Figure 3 shows the utility of total PSA, cPSA, and the free-to-total and complexed-to-total PSA ratios at the 95%, 90%, and 85% sensitivity levels in 604 men with total PSA between 2.0 and 10.0 ng/mL. Note the enhancement of specificity of cPSA over total PSA. Data in this PSA range is depicted because this is where the majority of patients who are being evaluated for possible biopsy of cancer in a screening cohort would fall (Figure 4).[16]

Should the PSA Cutoff Point for a Diagnosis of Prostate Cancer Be Lowered?

Georg Bartsch, MD, of the University of Innsbruck, examined the effect of lowering the PSA cut-off point for a diagnosis of prostate cancer. Capitalizing on his extensive Tyrollean screening project, he noted that only 64% of cancers detected in the total PSA range of 4–10 ng/mL were organ confined. This prompted a study of biopsies in men with lower PSA cut-

Table 2
cPSA Multicenter Prospective Trial Patient Demographic Data by Diagnosis

	Benign (n = 518)	Cancer (n = 310)	*P*
Age (y)	61 (55–68)	65 (59–71)	<.001
TPV (mL)	40 (28–59)	34 (26–47)	<.001
Total PSA (ng/mL)	3.8 (2.1–5.9)	5.5 (4.0–7.5)	<.001
cPSA (ng/mL)	3.0 (1.7–4.8)	4.6 (3.2–6.6)	<.001
% Free PSA	14 (10–20)	11 (8–16)	<.001
% cPSA	81 (75–87)	86 (81–91)	<.001

Data presented as mean (interquartile range). PSA, prostate-specific antigen; cPSA, complexed PSA; TPV, total prostate volume.
Data from Cheli et al.[15]

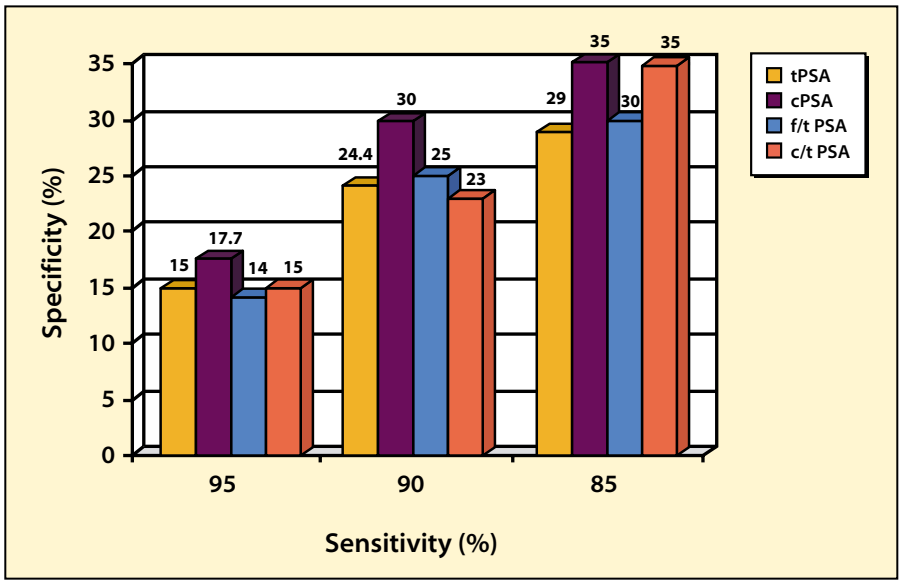

Figure 3. *Complexed prostate-specific antigen (cPSA) isoforms specificity at fixed sensitivity in total PSA (tPSA) range of 2.0–10.0 ng/mL (n = 604). f/t PSA, free-to-total PSA ratio; c/t PSA, complexed-to-total PSA ratio. Data from Cheli et al.*[15]

off points. In the ongoing trial in the Tyrol region of Austria, age-referenced PSA levels of 1.25–3.25 ng/mL are used in combination with percent free PSA less than 18%. In men with a PSA level between 4 ng/mL and 10 ng/mL, Dr. Bartsch uses a percent free PSA level of 27.5% or less as an indication for biopsy. In the lower total PSA range, he uses a 17.5% percent free PSA level as the cutoff point. He noted that doing this resulted in a significant increase in identifying men with cancer at a favorable pathologic stage (82.4% organ confined).

The concern for over-detection is obviously present. Although all men with a PSA level less than 2.0 ng/mL had organ-confined disease in the Tyrol experience, 4.2% of those with PSA between 2.0 and 4.0 ng/mL were shown to have positive margins. Clinically insignificant cancer, defined as that with a volume less then 0.2 mL and Gleason score less then 6, was 16.7%; 65% of men with PSA levels less than 4.0 ng/mL had multifocal tumors. Dr. Bartsch concluded that a high percentage of patients in whom the PSA level was low demonstrated tumors of significant malignant potential.

Difficulties in Pathologic Interpretation of Biopsy

Although elevation of PSA levels and, to a lesser extent, abnormality of digital rectal examination or other signs and symptoms might generate a suspicion for malignancy, the diagnosis of prostate cancer still demands pathologic interpretation of tissue specimens. Jonathan Epstein, MD, of Johns Hopkins University, reviewed the subject of prostate biopsy information critical for the urologist. He began his presentation by elucidating the potential for under-diagnosing prostate cancer. Variables, such as limited tissue on the needle biopsy, small areas of cancer on the needle biopsy, and subtle histologic abnormalities, all result in an often-times difficult situation for our pathologist colleagues.[17] Moreover, certain variants of prostatic carcinoma, such as pseudohyperplastic prostate cancer, foamy gland prostate cancer, atrophic prostate cancer, and cancers in men undergoing androgen deprivation, can all mimic benign conditions.

In addition, a number of nonmalignant lesions might resemble prostate cancer and lead to over-diagnosis. Dr. Epstein highlighted the utility of antibodies against high-molecular cytokeratin.[18,19] This follows from the observation that prostate cancer is

Figure 4. *Distribution of total prostate-specific antigen (tPSA) in screening (n = 1212). Percentages indicate patients with PSA levels in the range indicated by the corresponding bars. Reproduced with permission from Brawer.*[16]

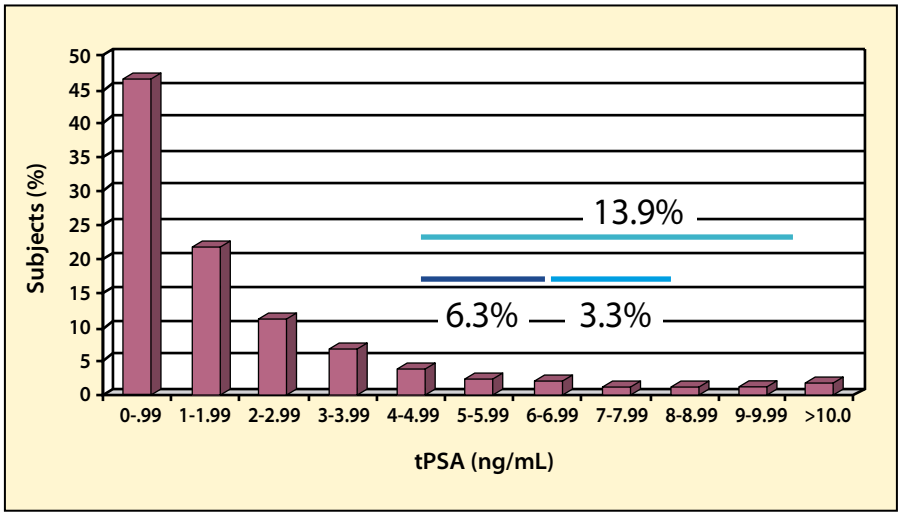

Table 3
Urologist- and Patient-Driven Second Opinions of Prostate Cancer Diagnosis

Outside Pathologist	Expert Review at Johns Hopkins University			
Benign (n = 42)	Benign 83.4%	Cancer 7.1%	Atypical 0%	HGPIN 9.5%
Cancer (n = 386)	Cancer 93%	Benign 1.5%	Atypical 5.5%	HGPIN 0%
HGPIN (n = 52)	HGPIN 75%	Atypical 11.5%	Cancer 5.8%	Benign 7.7%
Atypical (n = 204)	Atypical 36.8%	HGPIN 2.0%	Cancer 45.1%	Benign 16.1%

HGPIN, high-grade intraepithelial neoplasia.
Data from Epstein et al.[20]

devoid of basal cells, for which these stains are specific. He noted, however, that cytokeratin staining might not always be reliable. Rarely, benign glands do not stain positive, as in cases of adenosis, partial atrophy, and high-grade prostatic intraepithelial neoplasia, in which the basal cells might be attenuated in areas.

Dr. Epstein went on to discuss the clinical and economic impact of a second opinion for pathologic interpretation of needle biopsy specimens. From his experience with men scheduled for radical prostatectomy, 1.3% of needle biopsies that were interpreted as cancer by an "outside pathologist" were considered benign by Johns Hopkins investigators.[20] The comparison of outside pathology with the expert review at Johns Hopkins from this study is shown in Table 3. Based on these observations, Dr. Epstein made the comment that it is important to "know your pathologist." When in doubt, referral to pathologists who specialize in prostate cancer is warranted.

Dr. Epstein stated that when cancer is found on prostate needle biopsy, a number of parameters should be described, including the extent of the malignancy, perineural invasion, and grade.[21] The literature is confusing as to which modality of quantification of the extent of malignancy is best. These approaches include the number of positive cores, the fraction of positive cores, the total millimeters of cancer, the percent cancer per core, or the total percent of cancer. There is a strong correlation between the number of positive cores and extra-glandular extension.

One of the concerns of PSA testing has been the specter of over-detection. Epstein and colleagues[21] examined the radical prostatectomy specimens from 54 men with less-than-1-mm cancer that was Gleason score 3+3 in one core. On radical prostatectomy, 67% exhibited insignificant cancer tumor volumes of less than 0.5 mL and organ-confined disease with a Gleason score of 3+3 = 6. The authors demonstrated that using a PSA density cutoff point of less than 0.15 would identify 83% of patients with clinically insignificant tumors.

Finally, Dr. Epstein reviewed a number of predictors of malignant potential, including DNA ploidy, microvessel density, neuroendocrine markers, and proliferation assays. With respect to ploidy, he noted that this parameter correlates with pathologic stage and grade at radical prostatectomy; however, it does not add unique additional prognostic information beyond grade in predicting pathologic stage. Dr. Epstein showed that evidence regarding perineural invasion was confusing, with as many reports suggesting this as an independent predictor of extraprostatic extension as there were those suggesting that it has no utility.

The Role of Growth Factors in Prostate Cancer

With the increased likelihood of diagnosing tumors of low malignant potential, the need for reliable methods to differentiate these from more aggressive cancers is increasing. L. Michael Glodé, MD, of the University of Colorado, reviewed epidermal growth factor receptors in prostate cancer. He began his discussion by noting that the progression of prostate cancer from a hormonally dependent state to one of hormonal resistance is a multistep process involving numerous genetic and epigenetic changes.

Dr. Glodé noted that the key steps might include over-expression of specific cell membrane receptors and peptide growth factors expressed by the same cells (autocrine stimulation) or by the surrounding stroma (paracrine stimulation). He stated that in prostate cancer, growth factors including epidermal growth factor (EGF), transforming growth factor α, insulin-like growth factor-1, keratinocyte growth factor, platelet-derived growth factor, and basic fibroblast growth factor are thought to have a role. EGF is gaining

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increasing attention. EGF receptor (EGFR) has at least 4 entities, including Her2/neu. Her2/neu has been strongly linked to breast carcinoma.[22] With respect to prostate with Her2/neu, ErbB2 over-expression is inconclusive.[23,24] EGFR, also know as ErbB1, does seem to play a significant role in prostate cancer.[25] Dr. Glodé noted that among men treated with radical prostatectomy, 34 EGFR-positive patients relapsed, as compared with only 2 of 24 EGFR-negative patients.[25] This observation was particularly exciting given the numerous potential targets that exist for treating hormonally refractive prostate cancer through the EGFR signaling pathway.

A number of pharmaceutical companies are attempting to capitalize on these approaches. The University of Colorado has been evaluating a novel natural flavanoid, known as silibinin, which has been demonstrated to inhibit cancer cell division. Dr. Glodé noted that, at least in part, agents inhibit EGFR signaling.[26–29] In conclusion, Dr. Glodé noted that EGFR is emerging as an important therapeutic approach not only to prostate cancer but also to breast and colon cancer. ■

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Main Points

- Problems with total prostate-specific antigen (PSA) assay include its lack of sensitivity—there are false-negative results—but more importantly its lack of specificity, which results in a high number of false-positive test results. There is also concern regarding over-detection (detection of cancers that have limited malignant potential).
- An assay specific for complexed PSA (cPSA) has recently been developed. An evaluation of this assay demonstrated significant enhancement in test specificity, with approximately 20% improvement over that afforded by total PSA. A subsequent, expanded investigation also demonstrated improvement in test performance.
- A study of the effect of lowering the PSA cutoff point for a diagnosis of prostate cancer used a free PSA level of less than 18% in men with total PSA of 1.25 ng/mL to 3.25 ng/mL and a free PSA level of 27.5% or less in men with total PSA of 4 ng/mL to 10 ng/mL. This resulted in a significant increase in identifying men with cancer at a favorable pathologic stage (82.4% organ confined).
- Pathologic interpretation of tissue specimens can be problematic. Certain variants of prostatic carcinoma, such as pseudohyperplastic prostate cancer, foamy gland prostate cancer, atrophic prostate cancer, and cancers in men undergoing androgen deprivation, can all mimic benign conditions. Conversely, a number of nonmalignant lesions might resemble prostate cancer and lead to over-diagnosis.
- Growth factors including epidermal growth factor (EGF), transforming growth factor α, insulin-like growth factor-1, keratinocyte growth factor, platelet-derived growth factor, and basic fibroblast growth factor are thought to have a role in prostate cancer. EGF receptor, also know as ErbB1, seems to play a significant role.

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